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(71) Applicant *(for all designated States except US)*: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, 12th Floor, Oakland, California 94607 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CARSON, Dennis A. [US/US]; 9728 Keeneland Row, La Jolla, California 92037 (US). COTTAM, Howard B. [US/US]; 3144 Willow Creek Place, Escondido, California 92027 (US). WU, Christina C. N. [US/US]; 2392 Douglaston Glen, Escondido, California 92026 (US). LIVENGOOD, April J. [US/US]; 8666 Via Mallorca, La Jolla, California 92037 (US).

(74) Agents: KELLY, Beth L. et al.; Townsend and Townsend and Crew LLP, Two Embarcadero Center, 8th Floor, San Francisco, CA 94111-3834 (US).

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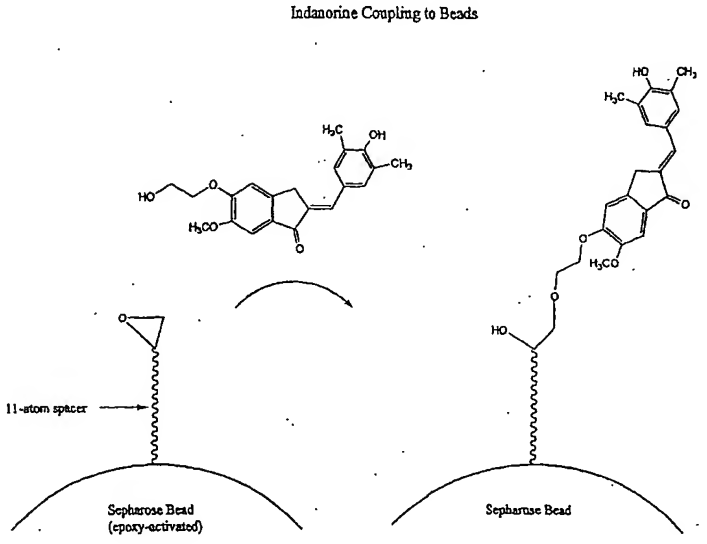

(57) Abstract: The disclosure provides pharmaceutical compositions comprising therapeutically effective compounds and nucleic acid drug enhancers. The disclosure also provides methods for identifying nucleic acid drug enhancers that specifically bind to therapeutically effective compounds. The invention also provides pharmaceutical compositions comprising therapeutically effective compounds, nucleic acid drug enhancers, and metal particle, e.g., gold particle. The pharmaceutical compositions comprising therapeutically effective compounds, nucleic acid drug enhancers, and metal particle are administered to subjects in need of treatment and the therapeutically effective compounds are released at an appropriate site in the body upon exposure to a radio-frequency magnetic field.

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ENCLOSING DRUGS WITH SYNTHETIC OLIGONUCLEOTIDES

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/566957, filed April 29, 2004 and claims the benefit of U.S. Provisional Application No. 60/621241, filed October 21, 2004; both of which are herein in incorporated by reference in their entirety for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under Grant No. 5 U01 AI56453, awarded by the National Institutes of Health/National Institute of Arthritis, Musculoskeletal, and Skin Diseases. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Aptamers are a relatively new class of therapeutic molecule that were first described 15 years ago. Aptamers are nucleic acid molecules that have been selected for the ability to bind tightly to a target molecule, such as a small organic molecule, protein, or peptide. Aptamers are isolated from a combinatorial library of oligonucleotides by a process known as SELEX (for systematic evolution of ligands by exponential enrichment) (Ellington, A. D. et al., *Nature* **346**:818-22 (1990); Tuerk, C. et al., *Science* **249**:505-10 (1990)). The library is easily generated by standard nucleic acid synthesis equipment. Approximately 10^{14}-10^{15} molecules can be screened simultaneously through sequential rounds of selection and amplification, leading to rapid identification of highly-specific binding molecules (Gold, L. et al., *Annu Rev Biochem* **64**:763-97 (1995)). Traditionally, aptamers themselves are used as drugs, for instance by binding to a protein and inhibiting its function. A prominent example is the VEGF binding aptamer developed for the treatment of macular degeneration.

[0004] Many drugs are effective in *in vitro* assays, but are not effective when tested in a patient for treating a condition of interest. For example, drugs that are insoluble, that bind to proteins, *e.g.*, serum proteins, tightly, or that are rapidly cleared from circulation are not effective for treating patients. Thus, there is a need for agents or methods to enhance the

pharmacokinetic properties of many drug candidates. The present invention solves this and other needs.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides a method of identifying a nucleic acid drug enhancer that specifically binds to a therapeutically effective compound, by contacting the therapeutically effective compound with a candidate drug enhancer, and detecting binding of the nucleic acid drug enhancer to the therapeutically effective compound, thereby identifying the nucleic acid drug enhancer. The nucleic acid drug enhancer is an oligonucleotide that has a secondary or higher order structure.

[0006] In one embodiment, the nucleic acid drug enhancer comprises a CpG motifs. CpG motifs can include the following sequences: 5' pur-pur-CpG-pyr-pyr 3', 5' pur-pyr-CpG-pyr-pyr 3', and 5' pur-pur-CpG-pyr-pyrCpG 3'.

[0007] In one embodiment, the nucleic acid drug enhancer comprises a guanine rich sequence.

[0008] In one embodiment, the nucleic acid drug enhancer comprises a nucleotide sequence that forms a structure selected from the group consisting of a guanine quartet, and an i-motif, thereby forming the higher order structure.

[0009] In one embodiment, the nucleic acid drug enhancer comprises a first nucleic acid molecule and a second nucleic acid molecule, and wherein the first nucleic acid molecule comprises a first nucleotide sequence comprising at least two nucleotides at the 5'-end or 3'-end that are complementary to a second nucleic acid sequence on the first or the second nucleic acid molecule, thereby forming the secondary structure.

[0010] In one embodiment, the nucleic acid drug enhancer comprises phosphorothioate linkages.

[0011] In one embodiment, the nucleic acid drug enhancer comprises phosphodiester linkages.

[0012] In one embodiment, the nucleic acid drug enhancer comprises chemically modified nucleotides.

[0013] In one embodiment, the nucleic acid drug enhancer is non-covalently bound to the therapeutically effective compound.

[0014] In one embodiment, the nucleic acid drug enhancer is covalently bound to the therapeutically effective compound.

[0015] In one embodiment, the nucleic acid drug enhancer is identified using SELEX. In another embodiment, the nucleic acid drug enhancer is identified through rational design by engineering a nucleic acid molecule comprising a nucleic acid sequence that is known to bind to a therapeutically effective molecule of interest.

[0016] In one embodiment, the therapeutically effective compound is selected from the group consisting of resiquimod, indanocine, indanorine, daunomycin, taxane, and adriamycin.

[0017] In one embodiment, the therapeutically effective compound binds to the nucleic acid drug enhancer by intercalation or minor groove binding. Therapeutically effective compounds that intercalate or exhibit minor groove binding include, *e.g.*, daunomycin, adriamycin, actinomycin D, distamycin.

[0018] In one embodiment, the therapeutically effective compound is a pharmacologically active known drug that has impaired in vivo activity because of poor water solubility, instability, and/ or excessive protein binding. Pharmacologically active known drug with impaired in vivo activity include, *e.g.*, indanocine, indanorine, taxol, acyclovir, and amphotericin B.

[0019] The present invention provides pharmaceutical compositions that comprise a nucleic acid drug enhancer that specifically binds to a therapeutically effective compound. The nucleic acid drug enhancer is an oligonucleotide that has a secondary or higher order structure. In one embodiment, the nucleic acid drug enhancer comprises a CpG motifs. CpG motifs can include, but are not limited to the following sequences: 5' pur-pur-CpG-pyr-pyr 3', 5' pur-pyr-CpG-pyr-pyr 3', and 5' pur-pur-CpG-pyr-pyrCpG 3'. In one embodiment, the nucleic acid drug enhancer comprises a guanine rich sequence. In one embodiment, the nucleic acid drug enhancer comprises a nucleotide sequence that forms a structure selected from the group consisting of a guanine quartet, and an i-motif, thereby forming the higher order structure. In one embodiment, the nucleic acid drug enhancer comprises a first nucleic acid molecule and a second nucleic acid molecule, and wherein the first nucleic acid molecule comprises a first nucleotide sequence comprising at least two nucleotides at the 5'-end or 3'-end that are complementary to a second nucleic acid sequence on the first or the second nucleic acid molecule, thereby forming the secondary structure. In one embodiment,

the nucleic acid drug enhancer comprises phosphorothioate linkages. In one embodiment, the nucleic acid drug enhancer comprises phosphodiester linkages. In one embodiment, the nucleic acid drug enhancer comprises chemically modified nucleotides. In one embodiment, the nucleic acid drug enhancer is non-covalently bound to the therapeutically effective compound. In one embodiment, the nucleic acid drug enhancer is covalently bound to the therapeutically effective compound. In one embodiment, the therapeutically effective compound is selected from the group consisting of resiquimod, indanocine, indanorine, daunomycin, taxane, and adriamycin. In one embodiment, the therapeutically effective compound binds to the nucleic acid drug enhancer by intercalation or minor groove binding. Therapeutically effective compounds that intercalate or exhibit minor groove binding include, *e.g.*, daunomycin, adriamycin, actinomycin D, distamycin. In one embodiment, the therapeutically effective compound is a pharmacologically active known drug that has impaired in vivo activity because of poor water solubility, instability, and/ or excessive protein binding. Pharmacologically active known drug with impaired in vivo activity include, *e.g.*, indanocine, indanorine, taxol, acyclovir, and amphotericin B.

[0020] The present invention also provides a method of administering a therapeutically effective compound to a subject, by contacting the therapeutically effective compound with an aptamer that specifically binds to the therapeutically effective compound, wherein the nucleic acid drug enhancer comprises a metal crystal, thereby making a heat releasable therapeutic agent; administering the heat releasable therapeutic agent to the subject; and applying a radio frequency magnetic field to an appropriate site on the subject, thereby releasing the therapeutically effective compound from the heat releasable therapeutic agent.

[0021] In one embodiment, the metal crystal is a gold crystal.

[0022] In one embodiment, the therapeutically effective compound is a chemotherapeutic agent. The chemotherapeutic agent can include, *e.g.*, resiquimod, indanocine, indanorine, and daunomycin. In a preferred embodiment, the chemotherapeutic agent is daunomycin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1 provides the structures of two immunostimulatory therapeutic compounds: imiquimod and resiquimod.

[0024] Figure 2 provides a reaction scheme for synthesis of a form of resiquimod (also referred to a R-848) that can be coupled to a solid support.

[0025] Figure 3 provides a reaction scheme for attachment of modified R-848 to a sepharose bead.

[0026] Figure 4 provides a reaction scheme for attachment of indanorine to a sepharose bead.

DETAILED DESCRIPTION OF THE INVENTION

[0027] The invention provides for the first time the use of aptamers, *e.g.*, nucleic acid drug enhancers, as a delivery system to improve the delivery of therapeutic compounds. In preferred embodiments, the nucleic acid drug enhancers or aptamers have a higher order structure, and therefore form aggregates around the therapeutic molecules. In further embodiments, the combination of the nucleic acid drug enhancers and the therapeutically effective molecule enhances a pharmacokinetic property, *e.g.*, solubility, stability, reduction of plasma protein binding, of the therapeutically effective molecule.

[0028] Aptamers have many features that make them excellent for therapeutic use. Like many small molecule drugs, they lack immunogenicity. In contrast, aptamers have a larger surface area and tend to fold into structures, so they can be designed to have greater specificity and binding affinity than small molecules (often nanomolar or even better) (Gold, L. et al., *Annu Rev Biochem* **64**:763-97 (1995)). This makes them an excellent alternative to antibodies, which are more expensive than aptamers on a per dose basis (Thiel, K et al., *Nature Biotechnology* **22**:649-651 (2004)). Furthermore, due to the chemical properties of nucleotides versus amino acids, aptamers are more stable and more easily produced than antibodies. Advances in the development of other nucleic acid therapeutics such as antisense and ribozymes have led to the identification of various modifications to enhance the half-life of nucleic acids in serum, which is particularly important for aptamers comprised of RNA, which are much less stable than aptamers comprised of single-stranded DNA (Brody, E. N. et al., *J Biotechnol* **74**:5-13 (2000)). DNA aptamers with and without terminal modifications have been shown to be stable in biological fluids (Jayasena, S. D. *Clin Chem* **45**:1628-50 (1999)). Additionally, DNA aptamers containing G-rich sequences capable of forming secondary structures and aggregates (*e.g.*, G-quadruplexes) have improved stability to nuclease, and increased uptake into certain cell types, such as B cells (Wu, C. C. et al., *J Biol Chem* **279**:33071-8 (2004); Liang, H. et al., *Curr Top Microbiol Immunol* **247**:227-40 (2000)).

[0029] Many therapeutically effective compounds cannot be administered systematically because they are (1) insoluble, (2) protein bound, (3) rapidly cleared from the circulation, or (4) non-specifically toxic to normal tissues. This disclosure provides a method for the preparation and use of nucleic acid drug enhancers, *e.g.*, oligonucleotides or aptamers, that specifically bind to therapeutically effective compounds, thereby increasing their solubility, and/or decreasing their protein binding, and/or prolonging their half-life. It should be noted that therapeutically effective compounds includes compounds that have an *in vitro* activity that indicates the potential for therapeutic benefit when administered to a patient.

Definitions

[0030] The term "nucleic acid" as used herein refers to a deoxyribonucleotide or ribonucleotide in either single- or double-stranded form. The term encompasses nucleic acids, *i.e.*, oligonucleotides, containing known analogues of natural nucleotides which have similar or improved binding properties, for the purposes desired, as the reference nucleic acid. The term also encompasses nucleic-acid-like structures with synthetic backbones. DNA backbone analogues provided by the invention include phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, morpholino carbamate, and peptide nucleic acids (PNAs); *see*, Oligonucleotides and Analogues, a Practical Approach, edited by F. Eckstein, IRL Press at Oxford University Press (1991); Antisense Strategies, Annals of the New York Academy of Sciences, Volume 600, Eds. Baserga and Denhardt (NYAS 1992); Milligan (1993) J. Med. Chem. 36:1923-1937; Antisense Research and Applications (1993, CRC Press). PNAs contain non-ionic backbones, such as N-(2-aminoethyl) glycine units. Phosphorothioate linkages are described in WO 97/03211; WO 96/39154; Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197. Other synthetic backbones encompassed by the term include methyl-phosphonate linkages or alternating methylphosphonate and phosphodiester linkages (Strauss-Soukup (1997) Biochemistry 36: 8692-8698), and benzylphosphonate linkages (Samstag (1996) Antisense Nucleic Acid Drug Dev 6: 153-156). Chemical modifications of nucleic acid molecules are also included. Modifications include, but are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrogen bonding, electrostatic interaction, and functionality to the nucleic acid ligand bases or to the nucleic acid ligand as a whole. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications,

modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil; backbone modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine and the like. Modifications can also include 3' and 5' modifications such as capping. The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide primer, probe and amplification product.

[0031] The phrase "specifically (or selectively) binds to" refers to the binding, duplexing, or hybridizing of a therapeutically effective compound preferentially to or only to a particular nucleotide sequence under binding conditions when that sequence is present in a complex mixture (*e.g.*, total cellular or library DNA or RNA).

[0032] As used herein, "oligonucleotide" is used interchangeably with the term "nucleic acid" and includes RNA or DNA (or RNA/DNA) sequences of more than one nucleotide in either single strand or double-stranded form. A "modified oligonucleotide" includes at least one nucleotide residue with any of: an altered internucleotide linkage(s), altered sugar(s), altered base(s), or combinations thereof.

[0033] As used herein, an "aptamer" is a nucleic acid which binds to a non-nucleic acid target molecule or a nucleic acid target through non-Watson-Crick base pairing.

[0034] Also provided by the invention is a method of identifying an aptamer or nucleic acid drug enhancer that binds to a therapeutically effective compound using a process termed "Systematic Evolution of Ligands by EXponential enrichment" (the "SELEX process"). The SELEX process is a method for the in vitro evolution of nucleic acid molecules with highly specific binding to target molecules and is described in, *e.g.*, U.S. Pat. Nos. 5,475,096; 5,670,637; 5,696,249; 5,270,163; 5,707,796; 5,595,877; 5,660,985; 5,567,588; 5,683,867; 5,637,459; 5,705,337; 6,011,020; 5,789,157; 6,261,774; EP 0 553 838 and PCT/US91/04078, each of which is herein incorporated by reference for all purposes.

[0035] As used herein a "higher order structure" or "secondary structure" is a structure formed by bonds between two molecules or by non-adjacent subunits, *e.g.*, nucleic acid residues, of the same molecule. The bonds are frequently non-covalent bonds. With regard to nucleic acid molecules, *e.g.*, the aptamers or nucleic acid drug enhancers of the present invention, higher order structures can be formed by Watson Crick base pairing between complementary nucleic acid sequences, or can include non-Watson Crick base pairing, *e.g.*, G quartets or i-motifs. The formation of higher order structures is sometimes indicated by detection of aggregate formation of DNA molecules.

[0036] As used herein, a CpG motif is an unmethylated CpG containing sequence. In preferred embodiments, a CpG motif sequences are known and include, *e.g.*, 5' pur-pur-CpG-pyr-pyr 3', 5' pur-pyr-CpG-pyr-pyr 3', and 5' pur-pur-CpG-pyr-pyrCpG 3'. Other CpG motif sequences are known in the art and are included for use in the invention. See *e.g.*, Schetter and Vollmer, *Curr Opin Drug Discov Devel.* 2:204-10 (2004); Agrawal and, Kandimalla, *Ann N Y Acad Sci.* 1002:30-42 (2003); Kerkmann *et al.*, *J Immunol.* 170(9):4465-74 (2003); and Hartmann and Krieg, *J Immunol.* 164(2):944-53 (2000); each of which is herein incorporated by reference for all purposes. In some embodiments, CpG motifs are included in nucleic acid drug enhancers or aptamers to increase immunostimulation by the combination of therapeutically effective molecule and nucleic acid drug enhancers or aptamers.

[0037] The term "contact" or "contacting" is used herein interchangeably with the following: combined with, added to, mixed with, passed over, incubated with, flowed over, etc.

[0038] A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins or other entities which can be made detectable, e.g., by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide. The radioisotope may be, for example, ^{3}H, ^{14}C, $^{3}2P$, 35S, or ^{125}I. In some cases, particularly using antibodies against the proteins of the invention, the radioisotopes are used as toxic moieties, as described below. The labels may be incorporated into the nucleic acids, proteins and antibodies at any position. Any method known in the art for conjugating the antibody to the label may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); David et al., Biochemistry, 13:1014 (1974); Pain et al., J. Immunol. Meth., 40:219 (1981); and Nygren, J. Histochem. and Cytochem., 30:407 (1982). The lifetime of radiolabeled peptides or radiolabeled antibody compositions may be extended by the addition of substances that stablize the radiolabeled peptide or antibody and protect it from degradation. Any substance or combination of substances that stablize the radiolabeled peptide or antibody may be used including those substances disclosed in US Patent No. 5,961,955.

Daunomycin is a potent anti-cancer drug with poor pharmacologic properties and cardiotoxicity

[0039] Daunomycin (daunorubicin, Cerubidine®) is a very potent anti-tumor anthracycline antibiotic. Daunomycin has been used in chemotherapy for various forms of cancer, and is currently a standard form of treatment for lymphoma. However, this drug has certain drawbacks, many of which are shared with other small molecule chemotherapy drugs. Daunomycin is a very hydrophobic molecule with poor pharmacologic properties such as low solubility, instability, and serum protein binding. Furthermore, daunomycin, like the majority of small molecule chemotherapy drugs, can pass quickly through cell membranes upon administration and has no selectivity for cancer cells (Moses, M. A. et al., *Cancer Cell* 4:337-41 (2003)). This results in adverse effects on normal tissues such as the heart, and thus limits the amount of drug that can be administered.

[0040] Improving the delivery of chemotherapeutics such as daunomycin is crucial for increasing efficacy and reducing toxicity, and can also improve pharmacologic properties. An alternative form of delivery is the liposomal encapsulation of daunomycin, or DaunoXome, used in treatment of AIDS-related Kaposi's sarcoma. Liposomes tend to accumulate in tumor tissues more than in normal tissues by virtue of the leaky vasculature that is characteristic of tumors; this has also been observed with small molecules linked to polymers (Moses, M. A. et al., *Cancer Cell* 4:337-41 (2003)). The cardiac safety of liposomal daunomycin remains unclear (Safra, T. Oncologist 8 Suppl 2:17-24 (2003)). Liposomes have promising features, but are difficult to make and are somewhat unstable and easily oxidized. Liposomes can accumulate and then be cleared by phagocytic cells of the reticuloendethelial system (Moses, M. A. et al., *Cancer Cell* 4:337-41 (2003)). Although liposomes clearly improve drug delivery, additional alternatives with increased targeting specificity and greater stability would be highly beneficial for improving anti-cancer drug efficacy.

Other therapeutically effective compounds

[0041] Therapeutically effective compounds or agents include small molecules, proteins, peptides, carbohydrates, steroids, and nucleic acids, *e.g.*, antisense molecules or RNAi molecules, that can be used to treat disease conditions or symptoms, or that are administered to improve health. Therapeutically effective compounds include anti-inflammatory agents, anti-cancer agents, antimicrobial agents, receptor agonists, receptor antagonists, anti-parkinsonian agents, antipsychotic agents, anti-depressants, anti-anxiety agents, anti-

analgesics, anti-seizure agents, anti-hypertension agents, agents for the treatment of asthma, anti-arrhythmic agents, agents for treatment of ulcers and reflux diseases, laxatives, anti-diarrheal agents, emetics, immunosuppressive agents, immunostimulatory agents, growth factors, cytokines, anticoagulants, hormones and hormone receptors. Many therapeutically effective compound are know to those of skill and are disclosed, *e.g.*, in Goodman & Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, Hardman and Limbird, eds., (1996); in Physicians' Desk Reference, Thompson (2004); and in The Merck Index, Merck & Co., Inc., Whitehouse Station, NJ, (2001); each of which are herein incorporated by reference.

[0042] In some embodiments the therapeutically effective compound is insoluble or unable to be solubilized to an extent necessary for administration to a patient.

[0043] In some embodiment the therapeutically effective compound is an aromatic or heterocyclic molecule capable of hydrogen bonding with a molecular weight between 100-1000 Daltons. One example of such a compound is Sumitomo compounds SM-360320 or its derivatives. Sumitomo compounds SM-360320 is known in the art. See, *e.g.*, Kurimoto *et al. Bioorg. Med. Chem.* 12:1091-1099 (2004) and US Patent No. 6,329,381, issued Dec. 11, 2001; both of which are herein incorporated by reference for all purposes.

[0044] In some embodiments, the therapeutically effective compound is an immunostimulatory agent. Two immunostimulatory agents, *i.e.*, imiquimod and resiquimod, are shown in Figure 1. Both of these agents are agonists of toll ligand receptors 7 and 8. Methods to assay for activity immunostimulatory agents such as imiquimod and resiquimod are found in U.S. Patent Application No. 10/824,833, filed April 14, 2004, which is herein incorporated by reference for all purposes. Another immunostimulatory agent is Sumitomo compounds SM-360320.

[0045] In some embodiments, the therapeutically effective compound is used to treat a cancer or other disease of proliferation. Many therapeutically effective compounds for cancer treatment are known to those of skill. Non-limiting examples of compounds for cancer treatment include indanocine, indanorine, and paclitaxil. Indanocine is described, *e.g.*, in U.S. Patent No. 6,162,810 and in Leoni *et al., J. Nat'l Cancer Inst.* 92:217-224 (2000), each of which is herein incorporated by reference. In other embodiments, the therapeutically effective compound is or a tubulin-binding drug such as a taxane, indanocine, or indanorine.

Aptamers and nucleic acid drug enhancers that specifically bind to therapeutically effective compounds

[0046] The present invention provides methods to identify aptamers, *e.g.*, nucleic acid drug enhancers that bind to therapeutically effective compounds. The invention also provides pharmaceutical compositions that comprise aptamers that bind to therapeutically effective compounds or nucleic acid drug enhancers that bind to therapeutically effective compounds.

[0047] A nucleic acid drug enhancer is a nucleic acid that specifically binds to a therapeutically effective compound and that enhances its use as a therapeutic. Enhancement of therapeutic use includes, *e.g.*, improved solubility of the therapeutically effective compound, decreased protein binding by therapeutically effective compound, decreasing non-specific cell binding, decreasing non-specific toxicity to normal tissues, and prolonging half-life of the therapeutically effective compound.

[0048] The aptamer or nucleic acid drug enhancer is, in some embodiments, a synthetic oligonucleotide from 10 to 100, or 150 base pairs long. In some embodiment the oligonucleotides are between 15 and 45 base pairs long. In preferred embodiments, the oligonucletides are between 20 and 40 base pairs long.

[0049] The nucleic acid drug enhancers or aptamers of the invention, in preferred embodiments, form a higher order structure. Formation of higher order structures can occur, *e.g.*, because of tertiary structural formation via G-quartet, stem loop structures, 3-way junction, etc., as well as i-motif formed by stretches of C, or because of the presence of guanine-rich sequences in the sequence, *e.g.*, at the 3', or 5' terminus of the oligonucleotide, or internally. G-quartet structures are formed by non-covalent bonds, *e.g.*, between guanine residues. I-motif structures are formed by non-covalent bonds, *e.g.*, between cytosine residues. For examples of such structures, see, *e.g.*, Phan and Mergny, *Nucl. Acids Res.* 30:4818-4625, (2002); which is herein incorporated by reference for all purposes. High order structures can also be formed by hybridization of complementary sequences within the same oligonucleotide molecule or between different oligonucleotide molecules.

[0050] Formation of higher order structure can easily be determined by those of skill. Formation of structures such as G-quartets and i-motifs can be determined using methods such as circular dichroism and NMR. See, *e.g.*, Wu *et al.*, *J Biol Chem.*, 279:33071-8 (2004) and Phan and Mergny, *Nucl. Acids Res.* 30:4818-4625, (2002); both of which are herein incorporated by reference for all purposes. Stem loop structure formation can be determined

by, *e.g.*, differential resistance to nucleases. Many of the aptamers or nucleic acid drug enhancers that form higher order structures form aggregates. Thus, formation of higher order structures such as G-quartets, stem loop structures, 3-way junctions, i-motifs, and hybridization of complementary sequences, can be determined by detecting aggregates, *e.g.*, by size exclusion chromatography or agarose gel electrophoresis.

[0051] In some embodiments the nucleic acid drug enhancers or aptamers that specifically bind to a therapeutically effective compound comprise a guanine rich region. A guanine rich region comprises at least 4 guanine residues, or in some embodiments, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20 guanine residues. The guanine rich region can be at the 5' terminus of the nucleic acid drug enhancers or aptamers, at the 3' terminus of the nucleic acid drug enhancers or aptamers, or internal.

[0052] In some embodiments, the nucleic acid drug enhancers or aptamers that specifically bind to the therapeutically effective compound comprise at least one CpG motif. In some embodiments an the therapeutically effective compound is an immunostimulatory agent and the nucleic acid drug enhancer or aptamer comprises a CpG motif.

[0053] Nucleic acid drug enhancers or aptamers that specifically bind to a therapeutically effective compound are identified as disclosed herein. In some embodiments, the nucleic acid drug enhancer or aptamer will be identified from a library of a large number of oligonucleotides. Those of skill will recognize that such libraries are commercially available, *e.g.*, from Integrated DNA Technologies of Corvallis Oregon. In a preferred embodiment the nucleic acid drug enhancers or aptamers comprise primer binding sites on at least one end to facilitate sequencing of the nucleic acid drug enhancer or aptamer.

[0054] The therapeutically effective compound of interest is coupled or attached to a solid support, such as a solid phase bead, via an aliphatic carbon chain of sufficient length, *e.g.*, 8-12 carbons. In some embodiments the coupling is performed using commercially available activated matrix, *e.g.*, epoxy-activated Sepharose 6B. Those of skill will recognize that many activated matrixes can be used in the invention, some of which are listed at biocompare.com/matrix/144/Activated-Media.html. The coupling reactions are conventional and known in the art.

[0055] Some therapeutically effective compounds will require modification before coupling to a solid support. Figure 2 provides a reaction scheme for synthesis of a form of

resiquimod (also referred to a R-848) that can be coupled to a solid support. Coupling of the modified R-848 and indanorine to a solid support are shown in Figures 3 and 4, respectively.

[0056] After coupling to a solid support, the therapeutically effective compound is contacted with the library of oligonucleotides, *e.g.*, candidate nucleic acid drug enhancers or aptamers that specifically bind to the therapeutically effective compound. After an appropriate period of incubation, the solid support is washed at low stringency to remove non-specifically binding oligonucleotides. The stringency of the washes is increased according to the needs of the user. A final high stringency wash, or alternatively, an affinity elution with the therapeutically effective compound, is performed to release the specifically binding oligonucleotides from the therapeutically effective compound. The specifically binding oligonucleotides are then sequenced, preferably using primers to sequences at one or both ends of the oligonucleotide. A description of oligonucleotide selection and sequencing is found in Wu *et al*, Human Gene Therapy, 14:849-860 (2003), which is herein incorporated by reference for all purposes. One or more oligonucleotides, *e.g.*, nucleic acid drug enhancers or aptamers, that bind to a therapeutically effective compound can be identified in this way.

[0057] In other embodiments, the nucleic acid drug enhancer or aptamer that binds to a therapeutically effective compound is developed and identified through rational design by engineering a nucleic acid molecule comprising a nucleic acid sequence that is known to facilitate binding to a therapeutically effective molecule of interest. In such a case, a the nucleic acid molecule is modified by addition, substitution, or deletion to add one or more nucleic acid sequence that facilitates formation of a higher order structure to form a nucleic acid drug enhancer or aptamer that comprises the nucleic acid molecule that is known to bind to a therapeutically effective molecule of interest. In some embodiments, the nucleic acid drug enhancer comprises a nucleic acid sequence that facilitates binding to a therapeutically effective molecule of interest. Some therapeutically effective compounds are known to bind to DNA helices, *e.g.*, by intercalation or minor groove binding. The interaction with the DNA molecule can be facilitated or enhanced by the presence of, *e.g.*, a specific nucleic acid sequence, or by the presence of a specific nucleic acid structure, or a combination of the two. The nucleic acid structure, thus, can formed by more than one nucleic acid sequence. The nucleic acid drug enhancer or aptamer, or the therapeutically effective compound can be labeled to facilitate determination of binding. Assays for determination of binding affinity are known.

[0058] Once specifically binding oligonucleotides are identified, they are mixed with the therapeutically effective compound and the combination is tested *e.g.*, for enhancement of solubility of the therapeutically effective compound, for decreased protein binding by therapeutically effective compound, for decreased non-specific cell binding by the therapeutically effective compound, decreasing non-specific toxicity to normal tissues of therapeutically effective compound, and for prolonging half-life of the therapeutically effective agent. Enhancement is assayed by comparing to a therapeutically effective compound with the specifically binding oligonucleotides to the same therapeutically effective compound without the specifically binding oligonucleotides. Those specifically binding oligonucleotides that enhance one of the therapeutically effective compound properties listed above are nucleic acid drug enhancers.

[0059] The stability of free versus aptamer-bound therapeutically effective compound will be assessed by UV-vis spectroscopy at 480 nm wavelength to detect the compound or by HPLC analysis (Taatjes, D. J. et al., *J Med Chem* 40:1276-86 (1997)). The stability of the aptamer alone versus aptamer plus therapeutically effective compound will be determined by the addition of low concentrations of nuclease or incubation in serum followed by polyacrylamide denaturing gel analysis to determine the extent of degradation of a radiolabeled aptamer or by circular dichroism analysis. Aptamer-bound to therapeutically effective compound is expected to be more tightly folded and thus more nuclease-resistant. Furthermore, it is predicted that aptamer-bound to therapeutically effective compound will exhibit reduced binding to serum proteins. This will be tested by incubation of radioactive therapeutically effective compound [^{3}H(G)], plus and minus the aptamer, with human serum albumin followed by HPLC separation of bound from free drug.

[0060] In some embodiments, binding of a nucleic acid drug enhancer to a therapeutically effective molecule increases the stability of the therapeutically effective molecule as compared to unbound therapeutically effective molecule. The stability or half life of the therapeutically effective molecule can be assayed in vitro, or in vivo, *e.g.*, in an appropriate animal system. The increase in half life of the nucleic-acid-drug-enhancer-bound therapeutically effective molecule can be, *e.g.*, at least 2, 3, 4, 5, 10, 20, 50, or 100 times greater than the half life of unbound nucleic acid drug enhancer.

[0061] In some embodiments, binding of a nucleic acid drug enhancer to a therapeutically effective molecule increases the solubility of the therapeutically effective molecule in

aqueous solution as compared to unbound therapeutically effective molecule in aqueous solution. The solubility of the therapeutically effective molecule can be assayed in vitro, or in vivo, *e.g.*, in an appropriate animal system. The increase in solubility of the nucleic-acid-drug-enhancer-bound therapeutically effective molecule can be, *e.g.*, at least 2, 3, 4, 5, 10, 20, 50, or 100 times greater than the solubility of unbound nucleic acid drug enhancer in aqueous solution.

[0062] In some embodiments, binding of a nucleic acid drug enhancer to a therapeutically effective molecule decreases the ability of the therapeutically effective molecule to bind to plasma proteins as compared to the ability of unbound therapeutically effective molecule to bind to plasma proteins. Plasma proteins include, *e.g.*, serum albumin and α-1 acid glycoprotein. The ability of the therapeutically effective molecule to bind to plasma proteins can be assayed in vitro, or in vivo, *e.g.*, in an appropriate animal system. The decrease in plasma protein binding of the nucleic-acid-drug-enhancer-bound therapeutically effective molecule can be, *e.g.*, less than 90%, 75%, 50%, 25%, 10%, 5%, 1%, or 0.1% of plasma protein binding by unbound nucleic acid drug enhancer.

[0063] Next, the cytotoxicity of aptamer-bound therapeutically effective compound is tested in an appropriate model system. Test can be done using tissue culture cells for some drugs. For example, for chemotherapeutics, aptamer-bound therapeutically effective compound can be added to transformed cell lines and the effect on cell proliferation can be determined. Studies to test the half-life and distribution of aptamer-bound therapeutically effective compound can be done in model organism, such as rodents using radioactively labeled aptamers and/or therapeutically effective compound

[0064] Since multiple specifically binding nucleic acid drug enhancers or aptamers can be identified using the above methods, it is expected that a single specifically binding nucleic acid drug enhancer or aptamer or a combination of specifically binding nucleic acid drug enhancers or aptamers can be used to enhance a property of a therapeutically effective compound. The present invention includes therapeutically effective compounds with one or multiple drug enhancers. The combination of a therapeutically effective compounds with one or multiple drug enhancers is administered to patient in need of treatment. The invention also includes kits that comprise a therapeutically effective compounds with one or multiple drug enhancers and instructions for use.

[0065] In some embodiments, the therapeutically effective compound binds to the nucleic acid drug enhancer by intercalation or minor groove binding. Preferably the nucleic drug enhancer will include regions of hybridization between complementary nucleic acid sequences. The hybridization can occur between complementary nucleic acid sequences on the same nucleic acid molecule, *e.g.*, the nucleic acid molecule will fold over on itself. Or the hybridization can occur between complementary nucleic acid sequences on the different nucleic acid molecules. Some known therapeutically effective compounds are known to intercalate into DNA helical structure or to bind to minor groove of a DNA helix, *e.g.*, daunomycin, adriamycin, actinomycin D, distamycin. Some DNA sequences that bind to intercalating drugs or minor groove binding drugs are known. See, *e.g.*, Ren, J. *et al.*, *Biochemistry* 38:16067-75 (1999); Bailly C. *et al., J Mol Recognit.* 5(4):155-71 (1992); and Brana, M.F. *Curr Pharm Des.* 7(17):1745-80 (2001); each of which is herein incorporated by reference for all purposes.

[0066] In some embodiments, the therapeutically effective compound is a pharmacologically active known drug that has impaired in vivo activity because of poor water solubility, instability, and/ or excessive protein binding. Such therapeutically effective compounds include, *e.g.*, indanocine, indanorine, taxol, acyclovir, and amphotericin B.

Nanoparticles can be tools for controlling the hybridization of nucleic acids

[0067] Nanotechnology is science and engineering involving materials, devices or systems on the nanometer scale, from a few to several hundred nanometers. A DNA helix has a diameter of approximately 2 nanometers and a protein molecule is approximately 5 nanometers long, whereas a cell is approximately 10 micrometers wide (10,000 nanometers) (Silva, G. A. *Surg Neurol* **61**:216-20 (2004); Salata, O. *J Nanobiotechnology* **2**:3 (2004)). Therefore, nanoparticles can be generated that are similar in size to biological molecules. Another advantage of nanoparticles is their size-dependent physical and chemical properties that can be manipulated with much greater precision than bulk materials (Silva, G. A. *Surg Neurol* **61**:216-20 (2004)).

[0068] The ability to use a nanoparticle to reversibly control the hybridization of a DNA oligonucleotide was shown by Hamad-Schifferli and colleagues (Hamad-Schifferli, K. et al., *Nature* **415**:152-5 (2002)). Gold nanocrystals, 1.4 nanometers in diameter, were covalently linked to DNA hairpin-loop oligonucleotides at a single position within the loop. Upon application of a radio-frequency magnetic field (RFMF), the nanoparticles were heated by

inductive coupling, which increases the local temperature of the DNA causing it to denature (disrupting the helical region). In the absence of the RFMF, the molecules returned to their original state. The temperature effect was very localized, as adjacent control molecules that lacked the nanoparticle were unaffected by the RFMF. The application of radio-frequency magnetic fields to patients in order to induce local heating of tumors, *i.e.*, radiofrequency ablation, is approved by the U.S. Food and Drug Administration for the treatment of cancer.

Aptamers and nanoparticle technology can be applied to drug delivery

[0069] Aptamers can be used, as described herein, to function as drug-enhancing agents that deliver small molecule chemotherapeutic drugs to selective sites. A major difficulty of related nucleic acids drugs such as antisense and ribozymes has been delivery into cells, requiring very high concentrations of aptamers to offset the low uptake. With our proposed approach, the aptamer does not have to be taken up into the cell because the small molecule is expected to diffuse easily through the cell membrane. DNA aptamers can be used, which have greater stability in serum than RNA aptamers. Identification of an aptamer that binds a small molecule drug, such as the anti-cancer drug daunomcyin, as described herein, is straightforward and rapid. Aptamers have been identified with excellent affinity for numerous classes of small molecule targets (Hermann, T. *et al.*, *Science* **287**:820-5 (2000)), but have not been used to deliver such molecules. Furthermore, small molecules have significantly more stable structures than alternative selection targets such as proteins (Gold, L. et al., *Annu Rev Biochem* **64**:763-97 (1995); Jayasena, S. D. *Clin Chem* **45**:1628-50 (1999)). Aptamers that bind to any therapeutically effective compounds listed herein can also be used as drug delivery agents.

[0070] A DNA aptamer will be selected to bind a therapeutically effective compound, *e.g.*, daunomycin. In some embodiments the aptamer is also a nucleic acid drug enhancer, and the therapeutically effective compound exhibits improved pharmacologic properties, such as increased stability and solubility in plasma in the presence of the aptamer. In a preferred embodiment, daunomycin is used.

[0071] Gold nanoparticles or other appropriate metal crystals, will be attached easily to the aptamers so that they can be transiently unfolded, and therefore release the therapeutically effective compound, upon exposure to a radio-frequency magnetic field (RFMF) near an appropriate sit in the subject. In one embodiment, the subject has a solid tumor and the RFMF is applied at or close to the site of a tumor. For haematologic cancers, RFMF is

applied to *e.g.*, lymph nodes, spleen, or thymus. RFMF has already been approved for use in cancer patients, and therefore is an acceptable technology for induced drug delivery. The ability to temporally and spatially control the release of daunomycin or other therapeutically effective compounds from the aptamer reduces the side effects of the drug.

Pharmaceutical compositions and administration

[0072] As noted above, aptamers or nucleic acid drug enhancer that bind to therapeutically effective molecules can be used to treat a variety of diseases. The aptamers or nucleic acid drug enhancer that bind to therapeutically effective molecules can be included in pharmaceutical compositions and can be administered by a variety of methods including, but not limited to parenteral (*e.g.*, intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes), topical, oral, local, or transdermal administration. These methods can be used for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include powder, tablets, pills, capsules and lozenges.

[0073] The compositions for administration will commonly comprise a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, *e.g.*, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

[0074] Thus, a typical pharmaceutical composition for intravenous administration would be about 0.1 to 10 mg per patient per day. Dosages from 0.1 up to about 100 mg per patient per day may be used, particularly when the drug is administered to a secluded site and not into the blood stream, such as into a body cavity or into a lumen of an organ. Substantially higher dosages are possible in topical administration. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are

described in more detail in such publications as *Remington's Pharmaceutical Science*, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980).

[0075] The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules and lozenges. It is recognized that antibodies when administered orally, should be protected from digestion. This is typically accomplished either by complexing the molecules with a composition to render them resistant to acidic and enzymatic hydrolysis, or by packaging the molecules in an appropriately resistant carrier, such as a liposome or a protection barrier. Means of protecting agents from digestion are well known in the art.

[0076] The compositions containing aptamers or nucleic acid drug enhancer that bind to therapeutically effective molecules of the invention can be administered for therapeutic or prophylactic treatments. In therapeutic applications, compositions are administered to a patient suffering from a disease (*e.g.,* cancer or immune disfunction) in an amount sufficient to cure or at least partially arrest the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of the agents of this invention to effectively treat the patient. An amount of an agonist that is capable of preventing or slowing the development of osteoarthritis in a patient is referred to as a "prophylactically effective dose." The particular dose required for a prophylactic treatment will depend upon the medical condition and history of the patient, the particular disease being prevented, as well as other factors such as age, weight, gender, administration route, efficiency, etc. Such prophylactic treatments may be used, *e.g.*, in a patient who is suspected of having a significant likelihood of developing osteoarthritis.

[0077] A "subject" for the purposes of the present invention includes both humans and other animals, particularly mammals. Thus the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the subject is a mammal, preferably a primate, and in the most preferred embodiment the patient is human.

[0078] It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" includes a plurality of such nucleic acids and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth.

[0079] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed. Citations are incorporated herein by reference.

EXAMPLES

1A. In vitro selection of daunomycin-binding aptamers

[0080] A phosphodiester deoxyribonucleic acid (DNA) library will be generated with a 40 nucleotide randomized region flanked on both ends with an approximately 20-nucleotide-primer-binding region (source, Integrated DNA Technologies). The library will contain approximately 10^{14} - 10^{15} molecules. For binding experiments, we will use daunomycin (Sigma) coupled to sepharose beads by virtue of the drug's free amine. The coupling reaction is conventional and is known in the art. The aptamer library will be incubated with daunomycin-sepharose in a physiologically relevant buffer, washed adequately, and bound aptamers will be eluted. Two elution strategies will be considered: competitive elution with free daunomycin or denaturing the nucleic acid. Eluted molecules will be amplified by asymmetric PCR to isolate a single strand as previously described (Wu, C. C. et al. *Hum Gene Ther* **14**:849-60 (2003)). This cycle will be repeated approximately 10 times, with intervening counterselection steps against sepharose beads without attached drug to select against aptamers that merely bind sepharose. Daunomycin, which intercalates into DNA, has a preference for GC base pairs with binding constants in the low micromolar range (Chaires, J. B. *et al., Biochemistry* **21**:3933-40 (1982)). Therefore it is anticipated that molecules in the library that form GC-rich helices with themselves or adjacent molecules will have moderate affinity for daunomycin. However, through successive rounds of selection, it is expected that the subset of molecules with the highest affinity for daunomycin will be isolated.

[0081] Once sufficient rounds of SELEX have been performed, the "winners" will be cloned into a vector and sequenced, then divided into families based on primary sequence similarities and/or predicted secondary structure similarities. The sequences will be aligned using standard alignment software such as Clustal W 1.8 (searchlauncher.bcm.tmc.edu/multi-align/multi-align.html). Additionally, secondary structure will be predicted using the freely available software mfold by M. Zuker (www.bioinfo.rpi.edu/applications/mfold/old/dna/form1.cgi). If the "winners" seem to be a random assortment of sequences with no obvious primary sequence or secondary structure motifs emerging, the selection can be redesigned and repeated, for example, by trying to increase the stringency of washes (*e.g.*, increase time, increase repetitions, alter ionic strength) or stringency of binding (*e.g.*, increase temperature, alter ionic strength).

1B. Identification of best aptamers for binding and increasing stability and solubility of daunomycin

[0082] One or two lead aptamers from each family generated by the selection will be synthesized without the flanking primer-binding sequence (which is usually not required for binding) (Jayasena, S. D. *Clin Chem* **45**:1628-50 (1999)), then analyzed using several assays to confirm that they exhibit desired properties. Equilibrium filtration, as previously described (Jenison, R. D. et al., *Science* **263**:1425-9 (1994)), will be used to determine the dissociation constant (K_d) of aptamers from each family for daunomycin. Thermal denaturation studies will be done to determine the T_m, the temperature at which 50% of the DNA is denatured, of aptamers in the presence or absence of daunomycin. A higher T_m, indicative of a more stable structure, is desirable. Comparison of T_m in the presence and absence of daunomycin for a given aptamer will elucidate if the aptamer has a more stable structure when bound to daunomycin.

[0083] To determine the minimal length requirement for the selected aptamers, mfold secondary structure predictions will be used to design a few shorter molecules that are predicted to retain the conserved structural features of the family of molecules from which they originate. These DNA oligonucleotides will be synthesized and then tested using competition dialysis to determine which of the truncated molecules have similar binding affinity to the full-length aptamer from which they were derived (Ren, J. et al., *Biochemistry* **38**:16067-75 (1999)). A few to several of these aptamers will be selected for further analysis, representating a range of dissociation constants and melting temperatures.

[0084] The stability of free versus aptamer-bound daunomycin will be assessed by UV-vis spectroscopy at 480 nm wavelength to detect daunomycin or by HPLC analysis (Taatjes, D. J. et al., *J Med Chem* 40:1276-86 (1997)). The stability of the aptamer alone versus aptamer plus daunomycin will be determined by the addition of low concentrations of nuclease or incubation in serum followed by polyacrylamide denaturing gel analysis to determine the extent of degradation of a radiolabeled aptamer or by circular dichroism analysis. Aptamer-bound daunomycin is expected to be more tightly folded and thus more nuclease-resistant. Furthermore, it is predicted that aptamer-bound daunomycin will exhibit reduced binding to serum proteins. This will be tested by incubation of radioactive daunomycin [^{3}H(G)] (source, Perkin Elmer), plus and minus the aptamer, with human serum albumin followed by HPLC separation of bound from free drug.

2A. Determine optimal sites of attachment of gold nanoparticles to aptamers

[0085] A single nanocrystal will be attached to the 3' or 5' end of aptamers by incubation of 1.4 nm diameter gold clusters (Nanoprobes) with DNA modified at either the 5' or 3' terminus with a free sulfhydryl group, as previously described (Alivisatos, A. P. *et al. Nature* 382:609-11 (1996), herein incorporated by reference). These AuNP-aptamers will first be tested in competition dialysis to verify that the affinity for daunomcyin has not been reduced. AuNP-aptamers that retain binding, and control aptamers (without AuNP), will be exposed to a RFMF. To assess drug release upon RFMF administration, biotinylated oligonucleotide AuNP-aptamers (or control aptamers without AuNP) will be attached to avidin sepharose and allowed to bind daunomycin [^{3}H(G)]. RFMF will be applied and then the extent of daunomycin release will be determined by measuring radioactivity in the supernatant following centrifugation.

[0086] If daunomycin-releasing aptamers are not identified in the previous experiments, structural information about the aptamers will be used in selecting sites for addition of gold nanoparticles. For example, chemical probing of nucleic acid structure, in the presence and absence of daunomycin, could yield information about which nucleotides are single-stranded and which are inaccessible either due to being base-paired or drug-bound. Gold nanocrystals will then be attached to internal, single-stranded regions adjacent to portions of the molecule that become protected upon drug binding using previously described methods (Hamad-Schifferli, K. *et al., Nature* 415:152-5 (2002)), and the aforementioned experiments will be repeated.

2B. Compare AuNP-aptamer for daunomycin delivery to liposomal daunomcyin in vitro and in vivo

[0087] Upon successful development of an AuNP-aptamer for delivery of daunomycin, *in vitro* and *in vivo* experiments will be performed to compare this drug delivery system to, *e.g.*, liposomal daunomycin (DaunoXome). The in vitro stability of DaunoXome (Gilead) versus AuNP-aptamer-bound daunomycin after incubation in serum will be determined. Next, the toxicity of aptamer-bound daunomycin versus DaunoXome to lymphoma cell lines maintained in the Carson lab will be determined. If AuNP-aptamer bound to daunomycin is sufficiently lethal to lymphoma cells in tissue culture, studies will be done to determine the half-life and distribution of aptamer-bound daunomycin (assaying for both daunomycin [^{3}H(G)] and aptamers) versus DaunoXome in vivo in mice. Ultimately, if results from the previous experiments are sufficiently promising, the efficacy of aptamer-bound daunomycin versus DaunoXome can be tested in a mouse lymphoma xenograft.

[0088] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A method of identifying a nucleic acid drug enhancer that specifically binds to a therapeutically effective compound, the method comprising the steps of

a) contacting the therapeutically effective compound with a candidate drug enhancer, and

b) detecting binding of the nucleic acid drug enhancer to the therapeutically effective compound, thereby identifying the nucleic acid drug enhancer;

wherein the nucleic acid drug enhancer has a higher order structure.

2. The method of claim 1, wherein the nucleic acid drug enhancer comprises a CpG motifs.

3. The method of claim 2, wherein at least one CpG motif has a sequence selected from the group consisting of 5' pur-pur-CpG-pyr-pyr 3', 5' pur-pyr-CpG-pyr-pyr 3', and 5' pur-pur-CpG-pyr-pyrCpG 3'.

4. The method of claim 1, wherein the nucleic acid drug enhancer comprises a guanine rich sequence.

5. The method of claim 1, wherein the nucleic acid drug enhancer comprises a nucleotide sequence that forms a structure selected from the group consisting of a guanine quartet, and an i-motif, thereby forming the higher order structure.

6. The method of claim 1, wherein the nucleic acid drug enhancer comprises a first nucleic acid molecule and a second nucleic acid molecule, and wherein the first nucleic acid molecule comprises a first nucleotide sequence comprising at least two nucleotides at the 5'-end or 3'-end that are complementary to a second nucleic acid sequence on the first or the second nucleic acid molecule, thereby forming the higher order structure.

7. The method of claim 1, wherein the nucleic acid drug enhancer comprises phosphorothioate linkages.

8. The method of claim 1, wherein the nucleic acid drug enhancer comprises phosphodiester linkages.

9. The method of claim 1, wherein the nucleic acid drug enhancer comprises chemically modified nucleotides.

10. The method of claim 1, wherein the nucleic acid drug enhancer is non-covalently bound to the therapeutically effective compound.

11. The method of claim 1, wherein the nucleic acid drug enhancer is covalently bound to the therapeutically effective compound.

12. The method of claim 1, wherein the nucleic acid drug enhancer is identified using SELEX.

13. The method of claim 1, wherein the nucleic acid drug enhancer is identified through rational design by engineering a nucleic acid molecule comprising a nucleic acid sequence that facilitates binding to a therapeutically effective molecule of interest.

14. The method of claim 1, wherein the therapeutically effective compound is selected from the group consisting of resiquimod, indanocine, indanorine, daunomycin, taxane, and adriamycin.

15. The method of claim 1, wherein the therapeutically effective compound binds to the nucleic acid drug enhancer by intercalation or minor groove binding.

16. The method of claim 15, wherein in the therapeutically effective compound is selected from the group consisting of daunomycin, adriamycin, actinomycin D, distamycin.

17. The method of claim 1, wherein the therapeutically effective compound is a pharmacologically active known drug that has impaired in vivo activity because of poor water solubility, instability, and/ or excessive protein binding.

18. The method of claim 17, wherein the pharmacologically active known drug is selected from the group consisting of indanocine, indanorine, taxol, acyclovir, and amphotericin B.

19. A method of administering a therapeutically effective compound to a subject, the method comprising:

a) contacting the therapeutically effective compound with an aptamer that specifically binds to the therapeutically effective compound, wherein the nucleic acid drug enhancer comprises a metal crystal, thereby making a heat releasable therapeutic agent;

b) administering the heat releasable therapeutic agent to the subject; and

c) applying a radio frequency magnetic field to an appropriate site on the subject, thereby releasing the therapeutically effective compound from the heat releasable therapeutic agent.

20. The method of claim 19, wherein the metal crystal is a gold crystal.

21. The method of claim 19, wherein the therapeutically effective compound is a chemotherapeutic agent.

22. The method of claim 19, wherein the aptamer is a nucleic acid drug enhancer that forms a higher order structure.

23. The method of claim 21, wherein the chemotherapeutic agent is selected from resiquimod, indanocine, indanorine, and daunomycin.

24. The method of claim 23, wherein the chemotherapeutic agent is daunomycin.

Resiquimod (R848)

No longer commercially avalable
more soluble than imiquimod
At least 10 times more potent IFNα inducer
TLR7/8 ligand

Imiquimod (R837)

Commercially available synthetic TLR7 ligand
Active ingredient in Aldara (5% cream)
Inducer of IFNα

FIG. 1

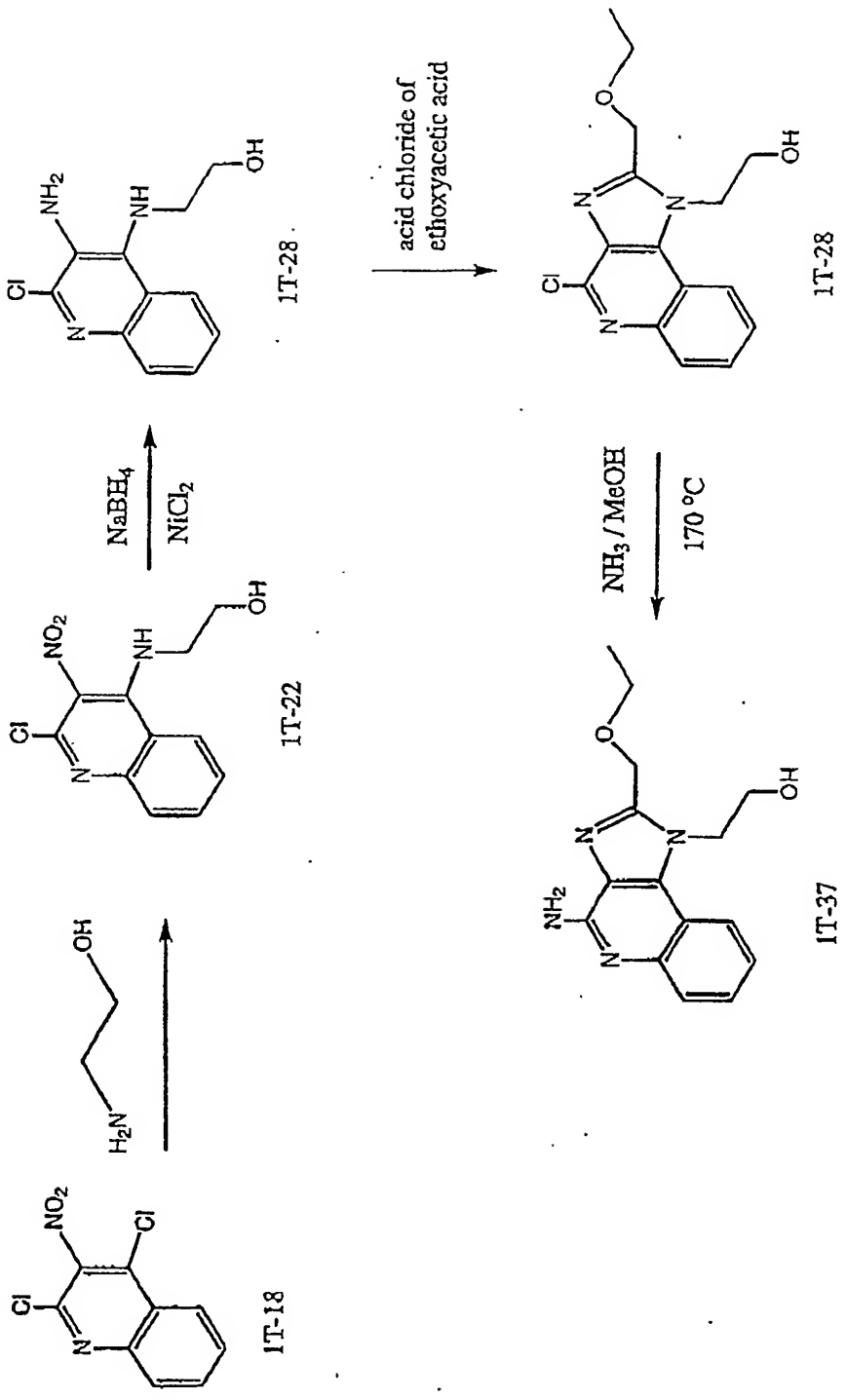
1T-18
1T-22
1T-28
1T-28
1T-37
NaBH4
NiCl2
acid chloride of ethoxyacetic acid
NH3 / MeOH
170 °C

FIG. 2

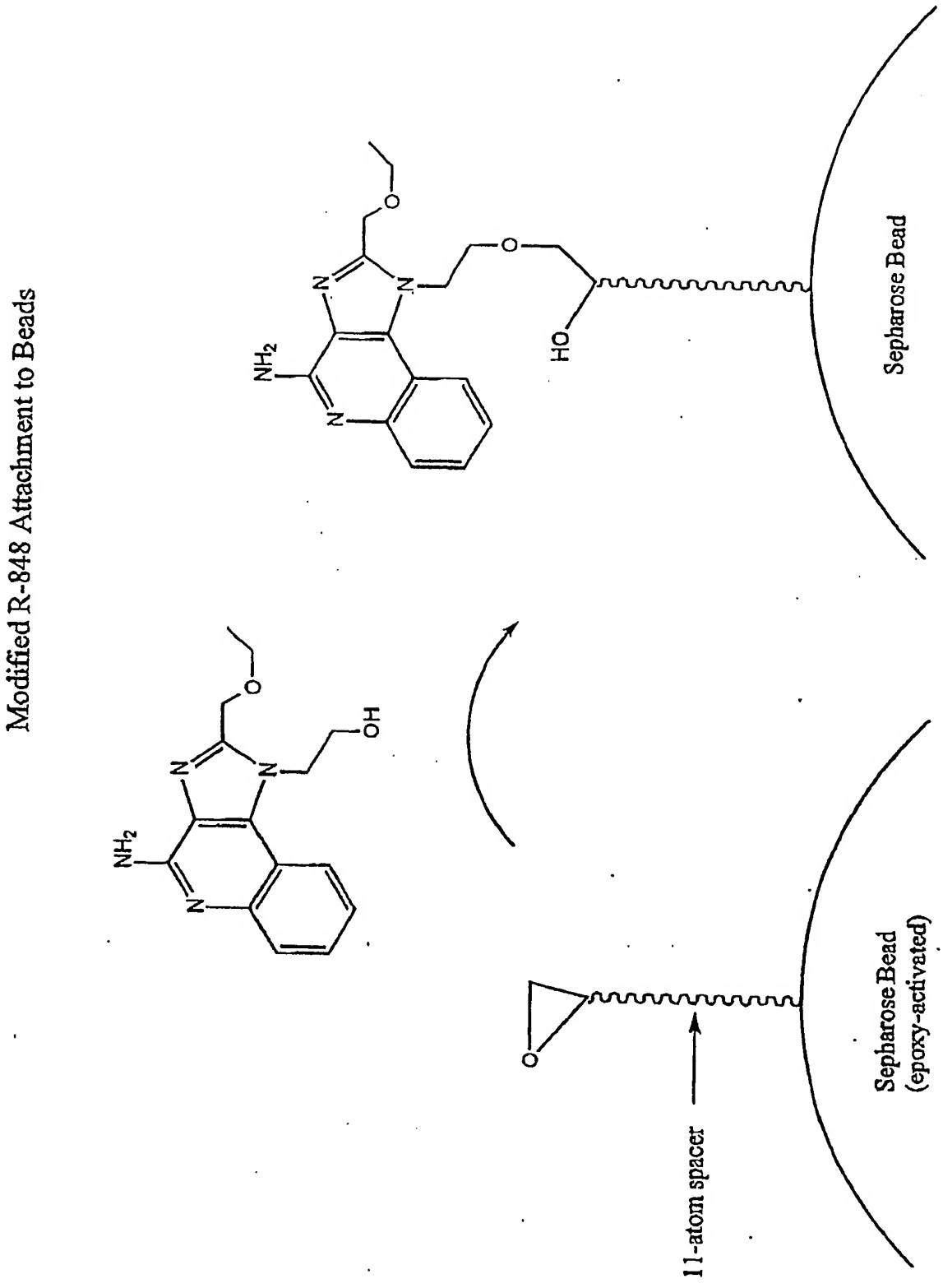
Modified R-848 Attachment to Beads
Sepharose Bead
HO
Sepharose Bead
(epoxy-activated)
11-atom spacer
OH

FIG. 3

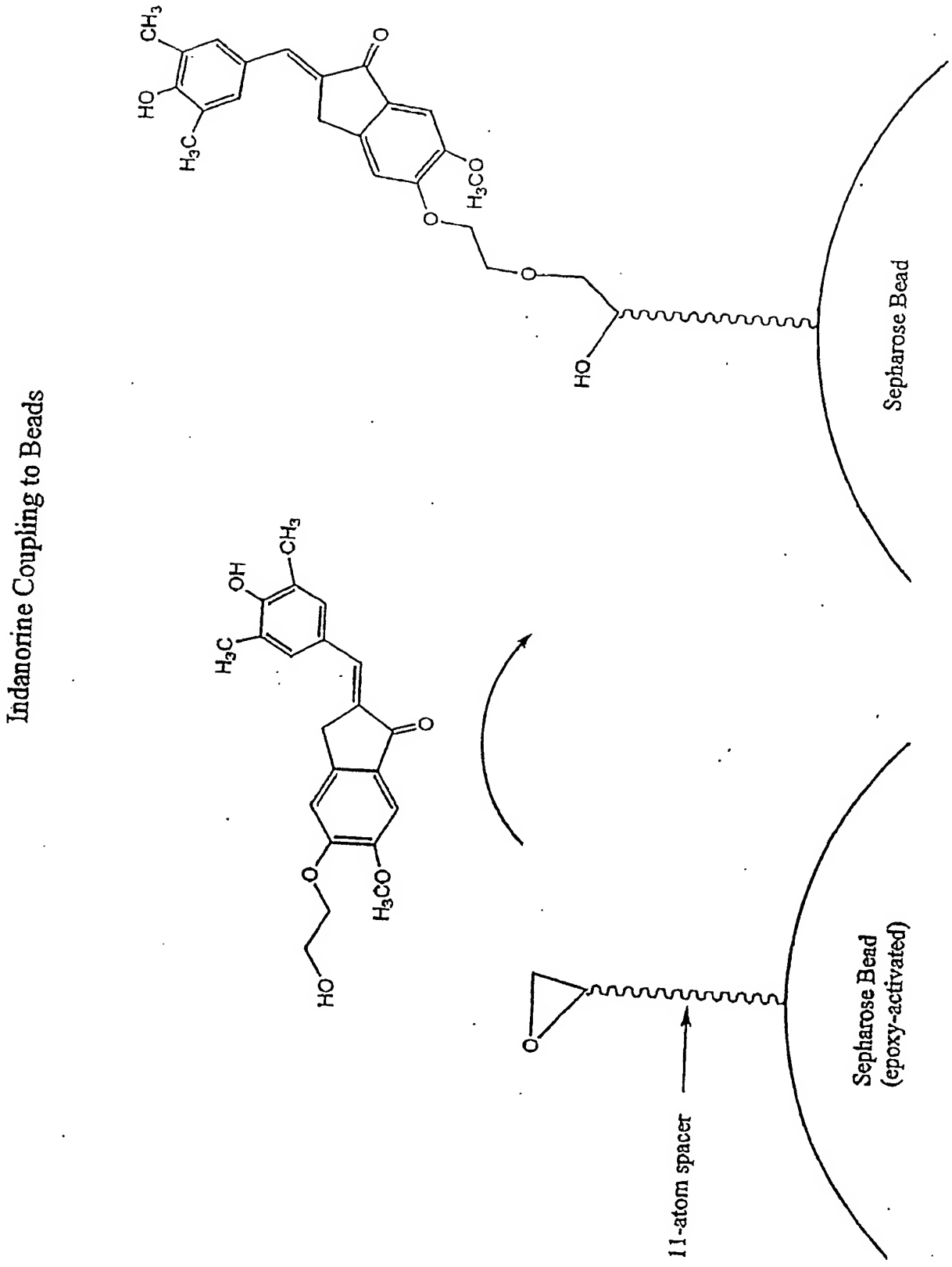
Indanorine Coupling to Beads
Sepharose Bead
Sepharose Bead
(epoxy-activated)
11-atom spacer

FIG. 4